## **WE CLAIM:**

- 1. A method of manufacturing electrochemical sensors, the method comprising steps of:
  - (a) forming a plurality of working electrodes on a first region of a substrate;
  - (b) forming a plurality of counter electrodes on a second region of the substrate;
  - (c) folding the substrate to overlay the first region and the second region;
  - (d) creating a sample chamber region between the first region and the second region; and
  - (e) separating a plurality of electrochemical sensors, each electrochemical sensor comprising at least one working electrode, at least one counter electrode, and at least one sample chamber region.
- 2. The method according to claim 1, wherein the step of folding the substrate comprises:
  - (a) scoring the substrate to form a score line; and
  - (b) folding the substrate along the score line.
- 3. The method according to claim 1, further comprising:
  - (a) positioning a spacer layer between the first region and the second region.
- 4. The method according to claim 3, wherein the step of positioning a spacer layer comprises:
  - (a) positioning an adhesive layer between the first region and the second region.
- 5. The method according to claim 4, further comprising:
  - (a) forming a plurality of indicator electrodes on the substrate in at least one of the first region and the second region; and

- (b) wherein the step of separating a plurality of electrochemical sensors comprises:
  - (i) separating a plurality of electrochemical sensors, each electrochemical sensor comprising at least one working electrode, at least one counter electrode, at least one indicator electrode, and at least one sample chamber region.
- 6. The method according to claim 1, wherein:
  - (a) the step of forming a plurality of working electrodes on a first region of a substrate comprises:
    - (i) forming a plurality of working electrodes on a first region of a substrate having a width and a length, the plurality of working electrodes arranged in columns parallel to the width and in rows parallel to the length; and
  - (b) the step of forming a plurality of counter electrodes on a second region of the substrate comprises:
    - (i) forming a plurality of counter electrodes on a second region of the substrate, the plurality of counter electrodes arranged in columns parallel to the width and in rows parallel to the length.
- 7. A method of manufacturing electrochemical sensors, the method comprising steps of:
  - (a) forming a plurality of working electrodes on a first substrate;
  - (b) forming a plurality of counter electrodes on a second substrate;
  - (c) forming a plurality of indicator electrodes on one of the first substrate and the second substrate;
  - (d) overlaying the first substrate and the second substrate to form a layered structure;
  - (e) creating a sample chamber region between the first substrate and the second substrate; and

- (f) separating a plurality of electrochemical sensors, each electrochemical sensor comprising at least one working electrode, at least one counter electrode, at least one indicator electrode, and at least one sample chamber region.
- 8. The method according to claim 7, wherein:
  - (a) the step of forming a plurality of working electrodes on a first substrate comprises:
    - (i) forming a plurality of working electrodes on a first region of the first substrate; and
  - (b) the step of forming a plurality of counter electrodes on a second substrate comprises:
    - (i) forming a plurality of counter electrodes on a second region of the first substrate.
- 9. The method according to claim 7, further comprising:
  - (a) forming a plurality of working electrode contact pads;
  - (b) forming a plurality of counter electrode contact pads;
  - (c) forming a plurality of indicator electrode contact pads; and
  - (d) wherein the step of separating a plurality of electrochemical sensors comprises:
    - (i) separating a plurality of electrochemical sensors, each electrochemical sensor having at least one working electrode contact pad, at least one counter electrode contact pad, and at least one indicator electrode contact pad.
  - 10. The method according to claim 9, wherein the step of separating a plurality of electrochemical sensors further comprises:
    - (a) cutting the second substrate to expose the working electrode contact pads,

- (b) cutting the first substrate to expose the counter electrode contact pads.
- 11. The method according to claim 10, wherein:
  - (a) the step of cutting the second substrate comprises:
    - (i) die cutting the second substrate; and
  - (b) the step of cutting the first substrate comprises:
    - (i) die cutting the first substrate.
- 12. The method according to claim 10, wherein after the steps of cutting:
  - (a) slitting the layered structure to provide individual electrochemical sensors.
- 13. The method according to claim 9, wherein:
  - (a) the step of forming a plurality of working electrode contact pads comprises:
    - forming a plurality of working electrode contact pads, each working electrode contact pad in electrical contact with one of the working electrodes;
  - (b) the step of forming a plurality of counter electrode contact pads comprises:
    - (i) forming a plurality of counter electrode contact pads, each counter electrode contact pad in electrical contact with one of the counter electrodes; and
  - (c) the step of forming a plurality of indicator electrode contact pads comprises:
    - (i) forming a plurality of indicator electrode contact pads, each indicator electrode contact pad in electrical contact with one of the indicator electrodes.

- 14. The method according to claim 7, further comprising:
  - (a) depositing an enzyme over a portion of the working electrodes or the counter electrodes.
- 15. The method according to claim 14, further comprising:
  - (a) depositing an enzyme continuously over a portion of at least two of the working electrodes or at least two of the counter electrodes.
- 16. The method according to claim 7, further comprising:
  - (a) depositing a redox mediator over a portion of the working electrodes or the counter electrodes.
- 17. The method according to claim 16, further comprising:
  - (a) depositing a redox mediator continuously over a portion of at least two of the working electrodes or two of the counter electrodes.